Chapter 3

Impact of the pre-intervention rate of renal function decline on benefit of renoprotective intervention

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ABSTRACT

The rate (slope) of long-term renal function loss is the best indicator of renal prognosis. For practical reasons however it is not usually applied as a parameter for inclusion or randomization in studies on long-term renal function loss. We hypothesized that the prior slope affects the outcome of these intervention studies.

We assessed the impact of pre-intervention renal function decline by analyzing pre-intervention renal function loss (during standard medical care) in 60 non-diabetic renal patients in whom an intervention study was performed (enalapril vs. atenolol), and a post-hoc analysis on ACE I/D polymorphism was carried out.

The pre-intervention slope correlated with the slope improvement during intervention ($r = -0.78$, $p<0.0001$), indicating better treatment benefit in patients with a steep slope before intervention. For enalapril and atenolol the slopes during intervention were similar. Despite a similar creatinine clearance at baseline however, pre-intervention slope was not well matched, i.e -3.7 ml/min/yr before enalapril versus -2.2 ml/min/yr before atenolol ($p=0.053$). A significant slope improvement was found during enalapril (intervention slope -1.9 ml/min/yr, $p<0.02$) but not during atenolol (intervention slope -1.8 ml/min/yr). In the analysis according to genotype the slope during intervention was significantly steeper in the DD genotype, suggesting treatment resistance. However, a significant improvement in slope was found only in DD genotype (-6.1 to 3.0 ml/min/yr, $p=0.001$), versus ID (-1.8 to -1.4 ml/min/yr) and II (-2.1 to -1.5 ml/min/yr). On multivariate analysis pre-intervention slope was the main predictor of slope improvement ($p<0.001$) and of the intervention slope ($p=0.005$), respectively.

Pre-intervention slope is the main determinant of renoprotective benefit, overruling the effect of specific pharmacologic intervention or genotype. Differences in pre-intervention slope can induce bias in intervention studies. For future intervention studies, allocation according to pre-intervention slope can preclude inclusions of non-progressors, and may allow to conduct more valid studies in smaller number of patients.

INTRODUCTION

In chronic renal disease renal function usually deteriorates progressively towards end stage renal failure. Major effort was made over the last decade to develop and evaluate renoprotective strategies by long-term intervention studies in man [1-7]. Whereas the rate of renal function loss is usually fairly constant for individual patients, it varies greatly between patients [8]. Theoretically, for studies on progressive renal function loss it would be best to include or randomize patients according to their prior rate of renal function loss, as the main predictor for renal prognosis. This could ensure an equal allocation of patients with high or low risk for progression to different treatment groups, and it could preclude inclusion of non-progressors. Not surprisingly, such approach has not been applied for comparative parallel trials, because of obvious practical problems in obtaining data on the rate of renal function loss before intervention. Therefore, patients are usually included according to baseline cross-sectional parameters, like renal function, blood pressure, and proteinuria as indirect indicators of renal prognosis.

We hypothesized that the prior rate of renal function loss might affect the outcome of studies on progressive renal function loss and that accordingly, neglect of this information could bias the interpretation of the results of intervention. To test this hypothesis, we analysed the impact of pre-study rate of renal function decline on the outcome of a 4-year prospective parallel intervention with atenolol versus enalapril [9]. In this prospective study progression rate was not different for the two regimens. In a post-hoc analysis of these data we found a faster progression rate in patients with ACE DD genotype [10]. Remarkably, the present analysis revealed that prior rate of renal function loss was the main determinant, not only of rate of renal function loss during intervention, but notably also of treatment benefit. Moreover, the interpretation of the data on the role of ACE genotype in treatment benefit was put into a quite different perspective.

Methods

In the previously published report, 89 patients with non-diabetic renal insufficiency were studied according to a prospective parallel randomized double-blind design [9]. In short, four weeks after withdrawal of antihypertensive drugs, baseline measurements were obtained. Inclusion criteria were a creatinine clearance of 30-90 ml/min and a diastolic blood pressure of more than 80 and less than 110 mmHg. Patients were randomized to treatment with atenolol or enalapril (starting dose 50/10 mg o.i.d. respectively). Drug dose was titrated on a goal diastolic blood pressure of 10 mmHg below baseline and/or below 95 mmHg. Patients were followed for three to four years.

For the present analysis, data on the period before study entry (during regular patient care) were retrospectively collected from patient records. Subjects were included if at least 1 year of follow-up during the pre-intervention period was available, with 3 or more data points for each parameter. At each visit, blood pressure was measured (during baseline and during the intervention period by automated device, Dynamap®), 24 hour urine was collected for determination of proteinuria (by pyrogallol red molybdate method) and creatinine. Blood was drawn for creatinine measurement (by standard autoanalyzer, SMA-C, Technicon®) to calculate creatinine clearance. Values were corrected for body surface area /1.73 m^2. ACE genotype was determined by PCR method using two different specific insertion primers to confirm putative DD genotypes and prevent mistyping as previously described [11].

Data on creatinine clearance, blood pressure and proteinuria are given as mean ± 95% CI during retrospective and prospective follow-up. The blood pressure and antiproteinuric responses were analysed as the change from baseline, that is, four weeks after withdrawal of prior treatment. The baseline characteristics of the original and the present analysis study group were compared by Chi-square test (gender, age, genotype, treatment and type of disease) and non-parametric Mann-Whitney U test (continuous variables). Differences between atenolol/enalapril and genotype groups were tested for slope, MAP and proteinuria by non-parametric ANOVA test. Mann-Whitney test was used to detect differences between two subgroups separately. Progression of renal function loss was estimated for each individual by calculating the slope of creatinine clearance versus time by the least squares regression method. The slope of renal function during the prospective study period is presented as the slope calculated from the data points as of three months onwards, to eliminate the influence of the initial effect of antihypertensive treatment on renal function [9].

Paired non-parametric Wilcoxon test was used to test the differences between blood pressure, proteinuria and slope, respectively, during the pre-intervention and the intervention period. Treatment benefit was expressed as slope improvement, i.e the difference between the pre-intervention slope and the intervention slope (delta slope) calculated by subtracting the pre-intervention slope from the intervention slope. In addition, the determinants of the intervention slope, and of delta slope, respectively, were analysed by multiple regression analysis. To this purpose, intervention slope and delta slope were modelled as respective outcome variables, with the pre-intervention slope and genotype as covariates, adjusting for baseline renal function, baseline and follow-up MAP and proteinuria. A two-sided p-value less than 0.05 was considered to be significant.

RESULTS

ACE genotype was obtained in 81 patients [10]. A total of 60 patients could be included in the present analysis. Mean follow-up was 59.2 ± 5.5 months. The other 21 patients all entered the prospective trial within one year after the diagnosis of renal disease. Thus, the pre-intervention period was too short to allow a valid slope assessment. Baseline characteristics in the present study sample were not significantly different from the original population (Table I).

Table I. *Patient characteristics before start of the intervention study, i.e. after withdrawal of previous medication (mean ± 95 % CI). Data are given for the original study and for the patients in the present analysis.*

	original study (n=81)	**present analysis (n=60)**
age (years)	49 ± 3	49 ± 3
creatinine clearance (ml/min)	55.1 ± 5.1	56.3 ± 6.0
MAP (mm Hg)	110 ± 3	108 ± 2
proteinuria (gr/day)	1.4 ± 0.5	0.9 ± 0.4
sodium excretion (mmol/day)	126 ± 17	127 ± 13
DD / ID / II (n)	17 / 37 / 27	15 / 24 / 21
use of antihypertensives, $\geq$ 1 (n)	57	41
diuretics (n)	20	15
beta-blockers (n)	32	23
ACE-inhibitors (n)	20	14
miscellaneous (n)	9	6
glomerulosclerosis/hypertension (n)	33	24
IgA nephropathy (n)	5	4
urolithiasis/reflux (n)	11	9
polycystic kidney disease (n)	11	9
miscellaneous (n)	21	14

Table II. *Rate of renal function loss, blood pressure and proteinuria during the pre-intervention and the intervention period (mean ± 95 % CI). Baseline renal function, blood pressure and proteinuria after 4 weeks washout (prior to intervention) are also given.*

	enalapril (n=31)	**atenolol (n=29)**	**DD (n=15)**	**ID (n=24)**	**II (n=21)**
pre-intervention period					
age (years)	50 ± 3	48 ± 3	49 ± 4	49 ± 2	49 ± 3
gender (male /female)	21/10	15/14	9/6	13/11	11/10
genotype (DD/ID/II)	9/ 11/11	6/13/10			
slope (ml/min/yr)	-3.7 ± 1.1	-2.2 ± 1.2	-6.1 ± 1.8*	-1.8 ± 1.0	-2.1 ± 1.1
mean MAP (mmHg)	108 ± 3	107 ± 4	110 ± 5	106 ± 4	108 ± 3
mean proteinuria (gr/day)	0.9 ± 0.4	1.0 ± 0.6	1.3 ± 0.9	1.0 ± 0.6	0.7 ± 0.4
baseline					
creatinine clear. (ml/min)	57.8 ± 10.1	56.9 ± 7.8	45.9 ± 11.9	50.1 ± 6.3	71.8 ± 10.4*
MAP (mmHg)	108 ± 3	111 ± 6	113 ± 5	106 ± 5	112 ± 5
proteinuria (gram/day)	1.1 ± 0.5	1.7 ± 0.8	1.9 ± 1.1	1.5 ± 0.9	1.1 ± 0.6
intervention period					
slope (ml/min/yr)	-1.9 ± 0.8#	-1.8 ± 0.7	-3.0 ± 0.8*/#	-1.4 ± 0.9	-1.5 ± 1.0
mean MAP (mmHg)	94 ± 3 $	96 ± 5 $	98 ± 5 $	94 ± 5 $	95 ± 4 $
mean proteinuria (gr/day)	0.5 ± 0.3 $	1.1 ± 0.6 $	1.1 ± 0.7 $	0.9 ± 0.6 $	0.5 ± 0.3 $

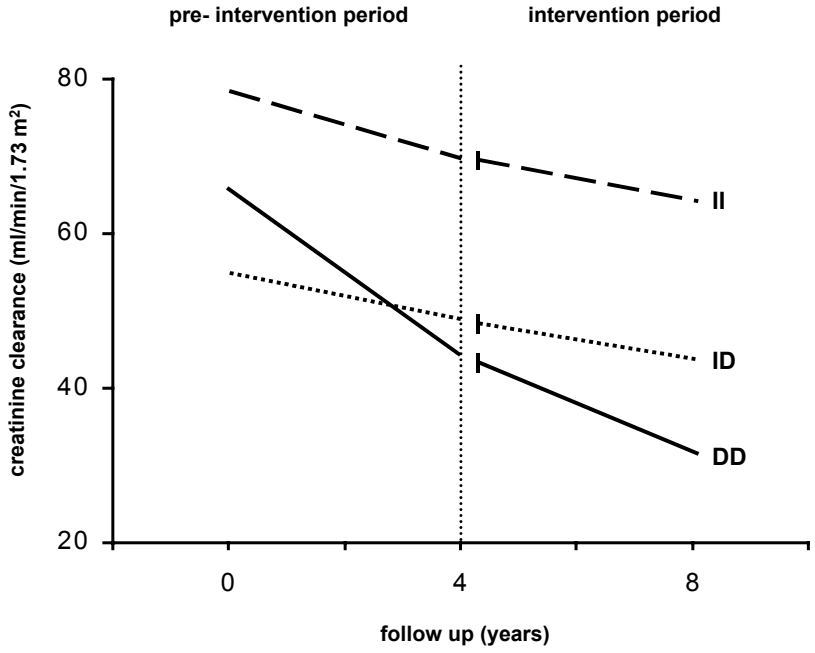
* p<0.05 compared to other genotypes / # p<0.05 compared to pre-intervention study period
$ p<0.05 compared to baseline.

For the groups randomized to enalapril and atenolol (Table II, left part) baseline creatinine clearance, MAP and proteinuria were not significantly different. During intervention, in accordance with our previous report, MAP, proteinuria and rate of renal function loss were similar with enalapril and atenolol [9]. However, the pre-intervention rate of renal function loss tended to be more rapid in the enalapril group, although the

difference did not quite reach statistical significance (p=0.053). Also, only the enalapril group had significantly better slope during intervention (p=0.018).

The corresponding data for a break-up according to ACE genotype (Table II, right part) reveal a higher creatinine clearance in the II genotype at baseline, with similar MAP and proteinuria for the three genotypes. During intervention, in accordance with our previous report, MAP and proteinuria were similar for the genotypes, with a worse rate of renal function loss in DD homozygotes [10]. Also, pre-intervention rate of renal function loss was significantly higher in the DD genotype. However, in the DD genotype, a significant benefit of intervention was apparent from the improvement in rate of renal function loss during intervention (p=0.0012, Figure 1). No such improvement was found in other genotypes.

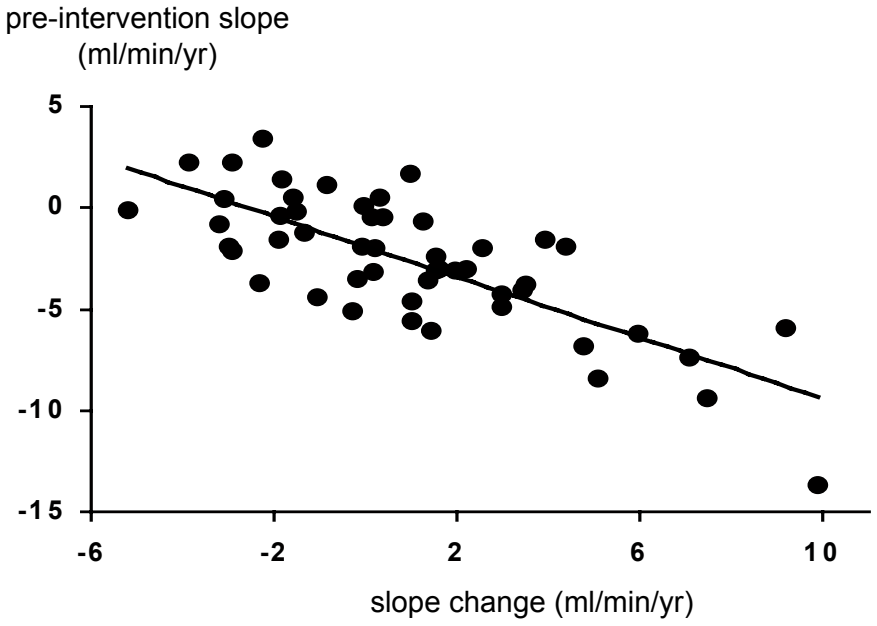
Figure 1. *Creatinine clearance slopes (ml/min/1.73 m^2) for DD, ID and II genotype during the pre-intervention and the intervention period. The pre-intervention slope is drawn by taking baseline renal function as reference and calculating renal function loss backwards. The intervention slope is calculated from three months on treatment to end of follow-up.*



For the population as a whole, the pre-intervention slope correlated with the improvement in slope during the intervention period (r= -0.78, p<0.0001, Figure 2), indicating a larger benefit of the trial regimen in patients with rapid renal function loss before intervention. A similar correlation was present for the enalapril (r= -0.72, p<0.0001) and atenolol (r= -0.64, p<0.0001) group and for each genotype group separately (DD; r= -0.90, p< 0.0001, ID; r= -0.66, p=0.0008, II; r= -0,71, p=0.0004).

On multivariate analysis the pre-intervention slope was an independent predictor of the intervention slope (P=0.005) and the delta slope (P<0.001), respectively, overruling the effect of ACE genotype, the change in MAP and proteinuria. If the pre-intervention slope was left out of the model, ACE DD genotype was an independent predictor of the intervention slope (P=0.048) and delta slope (P=0.041), respectively.

Figure 2. *Correlation between change in creatinine clearance slope (the intervention period slope minus the slope during the pre-intervention period) and the slope during the pre-intervention period (r= -0.78, p < 0.0001).*



DISCUSSION

This study demonstrates the impact of the pre-intervention rate of renal function decline, a neglected parameter in studies on long-term renoprotective intervention. We found that pre-intervention rate of renal function loss is a major predictor not only of the subsequent rate of renal function loss during intervention, but also of therapeutic benefit in terms of slope improvement.

Treatment benefit was largest in patients with rapid renal function loss before intervention and smallest or absent in those with a low renal risk to start with. Although this finding is intuitively obvious, it has never been formally demonstrated with respect to renoprotective intervention. This observation clearly parallels the findings in intervention studies in hypertension, where the a priori risk determines the benefit of antihypertensive treatment [12]. A post-hoc analysis from the MDRD study suggested that benefit of protein restriction depends on the underlying rate of renal function loss, without, however, providing data on rate of renal function loss without intervention [13].

We found that pre-intervention slope was the main predictor of the slope during intervention, and – on multivariate analysis - outweighed the effect of proteinuria and blood pressure. This might come as a surprise, considering the large body of evidence on the impact of baseline proteinuria and blood pressure as renal risk factors [1-4, 16]. It should be noted, however, that in other studies the predictive value of proteinuria and blood pressure was not tested for its independency from prior rate of renal function loss.

We used two outcome parameters, the intervention slope, and the delta slope, respectively. The delta slope is a derived variable with pre-intervention slope as a component. thus the possibility of bias due to mathematical coupling between pre-intervention slope and delta slope should be considered [14-15]. Mathematical coupling can induce bias mainly when accuracy and repeatability of measurements are low, and when the range of obtained values is small. In our analysis however, specific care was taken to obtain slope data with high accuracy and repeatability, by minimum requirements for number of data points and duration of pre-intervention period. Thus, a single slope value reflects multiple measurements (mean number of data points 11), which limits the risk that the observed relationship between pre-intervention slope and delta slope reflects mathematical coupling in stead of a pathophysiologically meaningful association. Whereas mathematical coupling cannot completely be excluded as a confounder for delta slope, pre-intervention slope was also the main predictor for the slope during intervention. As pre-intervention slope and intervention slope are mathematically independent, the conclusions of this analysis cannnot be biased by mathematical coupling.

We previously reported the similar rate of renal function loss during enalapril and atenolol [9]. The present analysis however, shows that randomisation to enalapril or atenolol according to creatinine clearance yielded an imperfect match for prior rate of

renal function loss, despite similar renal function, blood pressure and proteinuria at baseline. Whereas the difference in prior rate of renal function decline between the groups did not quite reach statistical significance - because of a wide scatter in individual slopes - this incomplete match may well have biased the estimation of treatment benefit from the parallel data. This is suggested by the significantly improved slope during intervention as compared to pre-intervention in the enalapril group only. As the intervention slopes were similar for the two regimens, the difference between the groups appears to be related to the slightly higher prior rate of renal function loss in the enalapril group.

The impact of prior rate of renal function decline was even more remarkable for the analysis of the effect of ACE genotype on study outcome. When assessed from the parallel intervention data only, the steeper slope in the DD genotype during intervention suggests lack of renoprotective benefit in these subjects. However, the pre-intervention data revealed that slope improvement was most readily apparent in DD homozygotes. This treatment benefit in DD subjects is in accord with other recent findings [15] and – according to our data – may be explained by their steeper pre-intervention slope rather than by ACE genotype as such, as slope improvement closely correlated with the pre-intervention rate of renal function decline independently of genotype. The steeper slope in DD genotype is in accord with several studies in diabetic and non-diabetic nephropathy [10, 17-22] but at variance with a recent study in proteinuric patients with non-diabetic renal disease [15]. In their study however, both proteinuria and renal prognosis were much worse than in our population, and the relative impact of phenotypic and genetic risk factors may be different under such circumstances [23].

The pre-intervention data in this study were obtained retrospectively. Thus, the potential flaws of a post-hoc analysis, and notably selection bias should be considered. From the original 81 patients, 21 could not be included. However, the only reason for non-inclusion of these patients was that the pre-intervention period was less than a year – precluding accurate assessment of the pre-intervention slope. Thus, no patients were lost to follow-up, and the intention-to-treat principle was not violated. Therefore, whereas we cannot exclude selection bias completely, we consider it unlikely that it plays a major role in the present results.

The predictive value of pre-intervention rate of renal function decline for treatment benefit is of clear relevance for future studies on renoprotective intervention. This holds particularly true for trials with relatively small numbers of patients, where inclusion of non-progressors may have a relatively large impact. Our data suggest that it would be fruitful to consider prior rate of renal function loss (if available) as a randomization parameter, as randomization on cross-sectional parameters may not warrant a sufficient match for the risk for renal function loss. Use of such longitudinal data may substantially enhance the power of studies to detect differences between treatment arms and thus reduce the required number of patients. For clinical purposes, it is important that our data show that a rapid rate of

renal function loss should not be considered a reason for therapeutic nihilism, but to the contrary identifies patients that particularly benefit from intervention treatment.
In conclusion, pre-intervention rate of renal function loss is a main determinant of subsequent renoprotective benefit. It may considerably affect the outcome and the interpretation of studies on chronic renal function loss. Considering prior rate of renal function loss as a randomization parameter, albeit cumbersome, may enhance study power and thus allow to conduct valid studies in smaller numbers of patients.

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